MAJOR ARTICLE

Modeling a Safer Smallpox Vaccination Regimen, for Human Immunodeficiency Virus Type 1–Infected Patients, in Immunocompromised Macaques

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We have modeled smallpox vaccination with Dryvax (Wyeth) in rhesus macaques that had depletion of $CD4^+$ T cells induced by infection with simian immunodeficiency virus or simian/human immunodeficiency virus. Smallpox vaccination induced significantly larger skin lesions in immunocompromised macaques than in healthy macaques. Unexpectedly, "progressive vaccinia" was infrequent. Vaccination of immunocompromised macaques with the genetically-engineered, replication-deficient poxvirus NYVAC, before or after retrovirus infection, was safe and lessened the severity of Dryvax-induced skin lesions. Neutralizing antibodies to vaccinia were induced by NYVAC, even in macaques with severe $CD4^+$ T cell depletion, and their titers inversely correlated with the time to complete resolution of the skin lesions. Together, these results provide the proof of concept, in macaque models that mirror human immunodeficiency virus type 1 infection, that a prime-boost approach with a highly attenuated poxvirus followed by Dryvax increases the safety of smallpox vaccination, and they highlight the importance of neutralizing antibodies in protection against virulent poxvirus.

The possible consequences of the deliberate release of smallpox by bioterrorists has led the United States to embark on a vigorous program to prepare new vaccines and to vaccinate at least a portion of its population [1]. The only currently available smallpox vaccine is a preparation of live vaccinia virus that had been used until routine vaccination was halted in 1972. In some immunocompromised individuals, Dryvax produced serious or even fatal complications, including "progressive vaccinia" (i.e., uncontrolled local enlargement of the skin lesion and dissemination of virus to other sites on the body) [2–6]. Cases of progressive vaccinia occurred either in infants with congenital immune defects or in adults with acquired immunodeficiency disorders, usually resulting from leukemia or lymphoma [7]. The continuation of Dryvax administration to military recruits resulted in a single reported case of disseminated vaccinia in a severely immunocompromised, human immunodeficiency virus (HIV)–infected soldier [8]. It is possible that more recruits with a lower degree of HIV-1–induced immunodeficiency have been exposed to Dryvax without developing overt complications [3].

Here, we tested the hypothesis that immunization of immunocompromised individuals, with highly attenuated poxviruses, may ameliorate the clinical outcome

Received 11 March 2003; accepted 5 May 2003; electronically published 10 October 2003.

Financial support: National Institutes of Health (contract N01-AI-15451 to M.G.L.).

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The Journal of Infectious Diseases 2003; 188:1181–91

0022-1899/2003/18808-0014

Table 1. Size of skin lesions in vaccinia-naive or -experienced immunocompetent and vaccinia-naive, immunocompromised macaques after Dryvax vaccination.

Group, macaque	Duration of lentivirus infection before Dryvax challenge (months)/virus	$CD4^+$ T cell count at time of Dryvax challenge, cells/mm^3	Area of maximum skin lesion after Dryvax challenge, cm^2
Group 1 (vaccinia naive)			
H684	NA	561	0.32
H461	NA	780	0.52
RH680	NA	1894	0.39
Mean ± SD	—	1078 ± 413	0.41 ± 0.06
Group 2[a] (NYVAC immunized)			
CA7R	*nef*$^-$ SIV_{mac239}	1286	0.233
AV2F	*nef*$^-$ SIV_{mac239}	912	0.026
CA7J	*nef*$^-$ SIV_{mac239}	798	0.0
Mean ± SD	—	999 ± 147	0.09 ± 0.07
Group 3 (vaccinia naive)			
3149	12/SIV_{mac251}	125	1.1
3228	12/SIV_{mac251}	100	⩾1.4
3176	12/SIV_{mac251}	84	—[b]
3222	8/SHIV 89.6 PD	38	1.2
3199	8/SHIV 89.6 PD	80	1.3
3200	8/SHIV 89.6 PD	0	NS
AV3B[c]	32/*nef*$^-$ SIV_{mac239}	422	0.73
Mean ± SD	—	121 ± 52	1.1 ± 0.11

NOTE. NA, not applicable; NS, not scanned; SIV, simian immunodeficiency virus.

[a] These macaques were infected with an SIV_{mac239} mutant in the *nef* gene and demonstrated normal $CD4^+$ T cell levels, as described elsewhere [15, 16].

[b] This macaque died of SIV infection at day 4.

[c] Macaque AV3B experienced a progressive decrease in $CD4^+$ T cells, as also observed in other macaques infected with *nef*$^-$ SIV_{mac239} [15, 16].

of Dryvax vaccination, by use of a macaque model of immunodeficiency. We chose as a vaccine the highly attenuated strain of vaccinia virus NYVAC, which originally was derived from a plaque-cloned isolate of the Copenhagen vaccine strain. In this strain, precise deletion of 18 open-reading frames (ORFs) has been generated, including the thymidine kinase (ORF J2R) and the large subunit of ribonucleotide reductase (ORF 14L) [9]. The attenuation characteristics of this strain have been demonstrated in in vitro and in vivo studies in immunocompromised and healthy rodents [9].

In macaques with modest to severe depletion of $CD4^+$ T cells, we tested whether immunization with NYVAC before or after infection with simian immunodeficiency virus (SIV) [10] or simian/human immunodeficiency virus (SHIV) [11] could increase the safety of Dryvax vaccination. We demonstrate that NYVAC was safer in severely immunocompromised macaques and that NYVAC priming resulted in a faster resolution of Dryvax-induced lesions in both healthy and immunocompromised macaques.

METHODS

Macaque history and Dryvax challenge. Twenty-five Indian rhesus macaques were enrolled in the present study. Institutional animal experimentation guidelines were followed. Six of the macaques were immunocompetent (groups 1 and 2; table 1), and macaques in group 1 were vaccinia naive. Macaques in group 2 had been exposed previously to the attenuated *nef*$^-$ SIV_{mac239} strain and were able to naturally control viral replication [12, 13] and maintain normal $CD4^+$ T cell counts. They were immunized with a single inoculation of NYVAC 1 month before Dryvax vaccination. Group 3 included 7 macaques, 3 that had been infected with the chimeric SHIV 89.6 PD strain for 8 months, 3 that had been infected with the SIV_{mac251} strain [12, 14] for 12 months, and 1 that had been infected with the *nef*$^-$ SIV_{mac239} strain for 32 months [15, 16] (table 1). This last macaque (AV3B) experienced a progressive decrease in $CD4^+$ T cell count, consistent with the findings of others [15, 16] (table 1). Most of the macaques in this group had severe de-

pletion of $CD4^+$ T cells and had $CD4^+$ T cell counts (mean ± SD, here and subsequently) of 121 ± 52 cells/mm^3.

Four macaques were inoculated with NYVAC 20 months before Dryvax vaccination and had been infected with SIV_{mac251} for 14 months (group 4; table 2). These macaques had $CD4^+$ T cell counts of 554 ± 103 cells/mm^3.

Four macaques (group 5; table 2) that, at first, had been infected with the same SIV_{mac251} strain [17, 18] and that subsequently were vaccinated with 3 inoculations of NYVAC, at weeks 10, 19, and 23 after infection (for macaques 480, 644, and H684) or at weeks 42, 48, and 54 after infection (for macaque 3143), were used (table 2). The overall time of SIV_{mac251} infection was 41 months, for macaques 480 and 644, and 25 months, for macaques H684 and 3143. All these macaques were viremic at the time of Dryvax vaccination and had $CD4^+$ T cell counts of 360 ± 131 cells/mm^3 (table 2). Four macaques (group 6; table 2) had been infected with SHIV 89.6 PD for 12 months and had blood $CD4^+$ T cell counts of 372 ± 160 cells/mm^3. They were vaccinated with 3 inoculations of NYVAC (10^8 pfu) 6 weeks apart and were challenged with Dryvax 6 months after the final NYVAC immunization.

All 25 macaques were vaccinated with Dryvax at the same dose at the times indicated in tables 1 and 2. In brief, the bifurcated needle was immersed in the vaccine suspension and was used to poke the skin 15 consecutive times, in accordance with US Food and Drug Administration (FDA) guidelines [4]. The lesions that developed after smallpox vaccination were photographed every 2 days and were imaged by manually defining the topographic contours of the affected skin.

Group 1 had significantly higher $CD4^+$ T cell counts than did group 3 ($P = .017$, Wilcoxon rank sum test). Groups 1 and 2 together had significantly higher $CD4^+$ T cell counts than did groups 3–6 together ($P = .0003$).

Imaging and statistical analysis. The area of the smallpox vaccination plaques was measured using a MetaMorph region measurements tool (Universal Imaging). Two-group comparisons of lesion sizes, times to resolution of lesions, and $CD4^+$ T cell counts were made by use of the exact Wilcoxon rank sum test, and correlations between them were tested by the Spearman's rank correlation method (StatXact Version 4.0.1; Cytel Software). A $CD4^+$ T cell count of 0 was assigned the value 10 when plotted on a logarithmic scale.

Neutralizing antibodies: β-galactosidase (Gal)–based vaccinia-neutralization assay. Plasma samples from representative macaques in groups 1–5 were collected immediately before Dryvax challenge (day 0) and after challenge at various

Table 2. Timing of NYVAC vaccination, simian immunodeficiency virus (SIV) infection, and size of skin lesions induced by Dryvax in vaccinia-experienced, immunocompromised macaques.

Group, macaque	Duration of infection before Dryvax challenge (months)/virus	Lag between the last vaccination with NYVAC and Dryvax challenge, months	$CD4^+$ T cell count at time of Dryvax challenge, cells/mm^3	Area of maximum skin lesion after Dryvax challenge, cm^2
Group 4[a]				
13M	14/SIV_{mac251}	20	797	0.38
11M	14/SIV_{mac251}	20	384	0.13
15M	14/SIV_{mac251}	20	383	0.28
20M	14/SIV_{mac251}	20	652	0.95
Mean ± SD	—	—	554 ± 103	0.44 ± 0.18
Group 5				
480	41/SIV_{mac251}	36	132	0.76
644	41/SIV_{mac251}	36	134	0.77
H684	25/SIV_{mac251}	20	611	0.98
3143	25/SIV_{mac251}	12	561	0.71
Mean ± SD	—	—	360 ± 131	0.8 ± 0.06
Group 6				
3051	12/SHIV 89.6 PD	6	173	1.04
3157	12/SHIV 89.6 PD	6	27	0.48
3164	12/SHIV 89.6 PD	6	616	0.46
3196	12/SHIV 89.6 PD	6	672	0.32
Mean ± SD	—	—	372 ± 160	0.58 ± 0.16

NOTE. SHIV, simian/human immunodeficiency virus.

[a] All macaques received 4 intramuscular vaccinations (10^8 pfu each) with wild-type NYVAC, except macaque 20M, which received NYVAC-SIV.

times (range, 7–40 days for different macaques). All plasma samples were heat inactivated at 56°C for 30 min and were evaluated for the presence of vaccinia-neutralizing antibodies by use of a novel assay based on expression of a reporter gene, β-Gal [19] . In brief, a recombinant vaccinia virus (vSC56) that expresses β-Gal under the control of a synthetic early/late promoter [20] was used to develop a neutralization assay based on a single-round infection of HeLa cells (CCL-2; ATCC). This is a rapid (24 h), high-throughput assay that has been shown to have similar sensitivity to the classic plaque reduction neutralization tests [19]. As a positive control, each assay includes FDA standard reference vaccinia immunoglobulin (Dynport Vaccine) vialed at the Center for Biologics Evaluation and Research (FDA, Bethesda, MD). Negative controls included plasma from unvaccinated children and albumin (5% solution; Alpha-Grifolf). Four serial dilutions of each macaque plasma sample were preincubated with vSC56 virus for 60 min at 37°C and then dispensed into 96-well, round-bottom plates containing 2×10^5 HeLa cells/well (4 replicates/antibody dilution). Plates were incubated for an additional 16 h at 37°C in a humidified CO_2 incubator. Cells then were lysed with the detergent IGEPAL CA630 (Sigma-Aldrich). In the second stage of the assay, β-Gal enzymatic activity in each well was measured using 96-well Immunlon 2 plates (Thermo Labsystems). Each plate included a β-Gal standard curve, which was derived by use of a recombinant β-Gal enzyme (Roche Diagnostics). Chlorophenol red β-D-galactopyranoside monosodium salt substrate (Roche Diagnostics) was added to all wells and was incubated for 30 min at room temperature in the dark, and the enzymatic reaction was stopped with 1 mol/L Na_2 CO_3 solution. Optical density was determined at 575 nm by use of an ELISA reader (Bio-Tek). Optical density readings were transferred to Microsoft Excel for further analysis. The β-Gal standard curves were used to convert optical density values into β-Gal activity per experimental or control group (in milliunits per milliliter). The β-Gal activity of each experimental group (virus mixed with a given dilution of test plasma) was expressed as the percentage of β-Gal activity in the virus-only control wells. Microsoft Excel was used to plot the percentage of control values for the serial dilutions of each plasma versus log dilutions. The equation of each curve was used to calculate the ID_{50} value.

RESULTS

Modeling NYVAC and smallpox vaccination in healthy macaques. Vaccination of 3 vaccinia-naive, healthy rhesus macaques with Dryvax resulted in skin lesions that became evident 4 days after inoculation (figure 1*A*), reached a mean size of 0.4 cm^2 within 10–12 days, decreased in size thereafter, and healed completely with loss of the scab within 20 days. The mean lesion size over time, obtained by imaging analysis, mimics that observed in humans (figure 1*B*) [4].

To assess the degree and durability of Dryvax-induced immunity, the same macaques (group 1) were vaccinated 2 months later with Dryvax. As in humans [4], the size of skin lesions in all these macaques was significantly smaller and the time to resolution was shorter (6 days) than before (figure 1*A* and 1*C*).

To assess whether immunization with NYVAC [21] would provide an equivalent degree of protection against Dryvax replication, immunocompetent macaques (figure 1*D;* table 1, group 2) were inoculated intramuscularly with 10^8 pfu of NYVAC [13]. As expected, no lesions were observed at the site of inoculation (data not shown). The same macaques were challenged 1 month later with Dryvax. The skin lesions and time to resolution (figure 1*A,* 1*D,* and 1*E;* table 1) were equivalent to those observed in macaques previously vaccinated with Dryvax (compare figure 1*C* with figure 1*D*). The mean lesion size for NYVAC-primed macaques is compared with that for vaccinia-naive macaques in figure 1*E*.

Dryvax-induced complications in immunocompromised macaques. Replication of Dryvax was assessed in macaques with severe $CD4^+$ T cell depletion ($CD4^+$ T cell count, 121 ± 52 cells/mm^3). These macaques had been infected with either the pathogenic chimeric SHIV 89.6 PD or the SIV_{mac251} strain (table 1, group 3). Infection with the SHIV 89.6 PD strain in rhesus macaques is associated with a high level of chronic viremia and a rapid decrease in $CD4^+$ T cells within a few weeks after infection [11]. In contrast, infection with the SIV_{mac251} strain, which also causes a high level of chronic viremia, is characterized by a slowly progressive loss of $CD4^+$ T cells and development of AIDS within 2–3 years after infection [10]. We used 3 macaques infected with SHIV 89.6 PD (figure 2*A*) and 3 macaques infected with SIV_{mac251} strain 561 [12] (figure 2*B*). An additional macaque, AV3B, which was infected with *nef*$^-$ SIV_{mac239} but nevertheless experienced $CD4^+$ T cell depletion [15, 16], was included.

Skin lesions became evident in these immunocompromised macaques between 4 and 6 days, as observed in healthy macaques (figure 1*A*). In contrast to those on healthy macaques, however, the skin lesions continued to enlarge after 10–12 days (figure 2*C*). The mean maximum lesion area in these macaques was ~3-fold larger than that observed in immunocompetent macaques (1.1 vs. 0.4 cm^2), and most macaques developed lesions of >1 cm^2 in size and experienced a delayed time to resolution (figure 2*C,* 2*D,* and 2*E;* table 1). Macaque 3228, which had a $CD4^+$ T cell count of 100 cells/mm^3 at the time of Dryvax vaccination, developed skin lesions that displayed signs of local invasiveness; when the skin lesion reached 1.4 cm^2, the macaque was killed (day 16) (figure 2*C*). The clinical diagnosis in this macaque was consistent with that of progres-

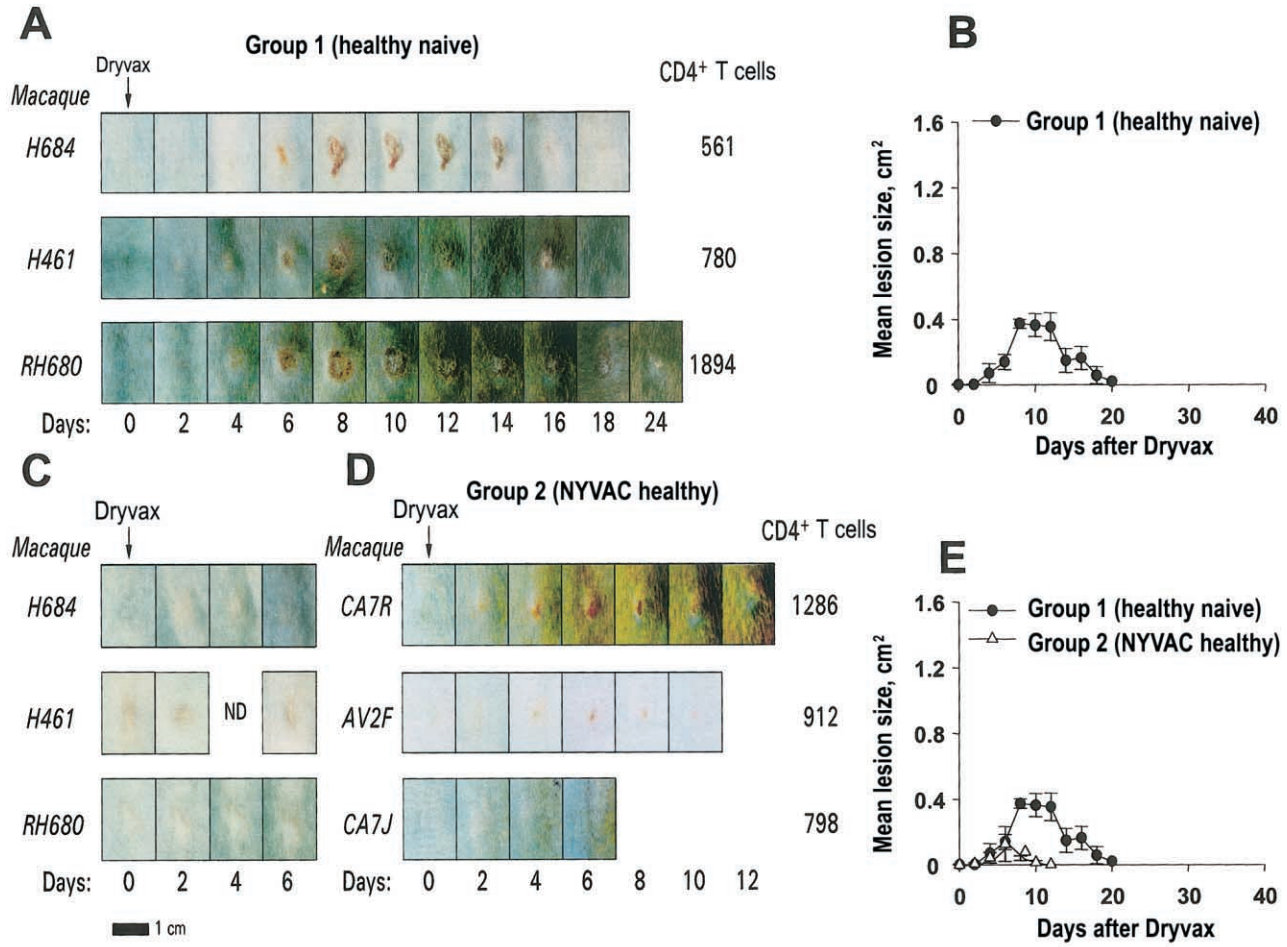

Figure 1. Dryvax-induced lesions in vaccinia-naive or -experienced immunocompetent macaques. *A,* Skin lesions induced by Dryvax in vaccinia-naive immunocompetent macaques. *B,* Mean lesion size obtained by imaging and time to resolution in macaques from panel *A. C,* Skin lesions in immunocompetent macaques from group 1 *(A)* vaccinated again with Dryvax, ~2 months from the first Dryvax inoculation. *D,* Macaques vaccinated 1 month before Dryvax challenge (group 2) with 1 intramuscular dose of NYVAC (10^8 pfu). *E,* Comparison of mean lesion size in macaques from groups 1 and 2 over time.

sive vaccinia. Macaque 3176 died of SIV disease 4 days after Dryvax vaccination (table 1) and could not be further evaluated. None of the macaques in this group developed neutralizing antibodies to vaccinia within the first 4 weeks after Dryvax vaccination.

Statistical analysis of the imaging-measured size of the lesion areas, by use of the Wilcoxon rank sum test, demonstrated that macaques in group 3 had significantly larger lesions than did the immunocompetent macaques in group 1 ($P = .036$) and that the time to resolution of skin lesions was delayed ($P = .071$). These data suggest that, in immunocompromised macaques, Dryvax replication is controlled less effectively and can cause life-threatening conditions, as observed in humans with immunodeficiency [8].

Resolution of Dryvax-induced lesions in vaccinia-experienced, SIV_{mac251}-infected macaques. Because the immunity conferred by previous vaccination with NYVAC resulted in smaller skin lesions and shortened time to resolution in immunocompetent macaques (figure 1*D* and 1*E;* table 1), we wished to evaluate whether that would also be the case in immunocompromised macaques. Four macaques, previously vaccinated with NYVAC (group 4) and subsequently infected with SIV [14, 22] (figure 3*A* and 3*B;* table 2), were exposed to Dryvax. These macaques experienced Dryvax-induced lesions, with a mean maximum area similar to that of immunocompetent macaques (table 2), and the size and time to resolution of skin lesions differed significantly from those observed in macaques in group 3 ($P = .032$ and $P = .024$, respectively, Wilcoxon rank sum test) (figure 3*C* and 3*D*). Because immunization of these macaques with NYVAC preceded Dryvax challenge by 20 months (table 2), it could be assumed that vaccinia immunity was preserved in these macaques despite the immunological damage induced by SIV_{mac251} infection.

NYVAC vaccination after onset of severe CD4+ T cell de-

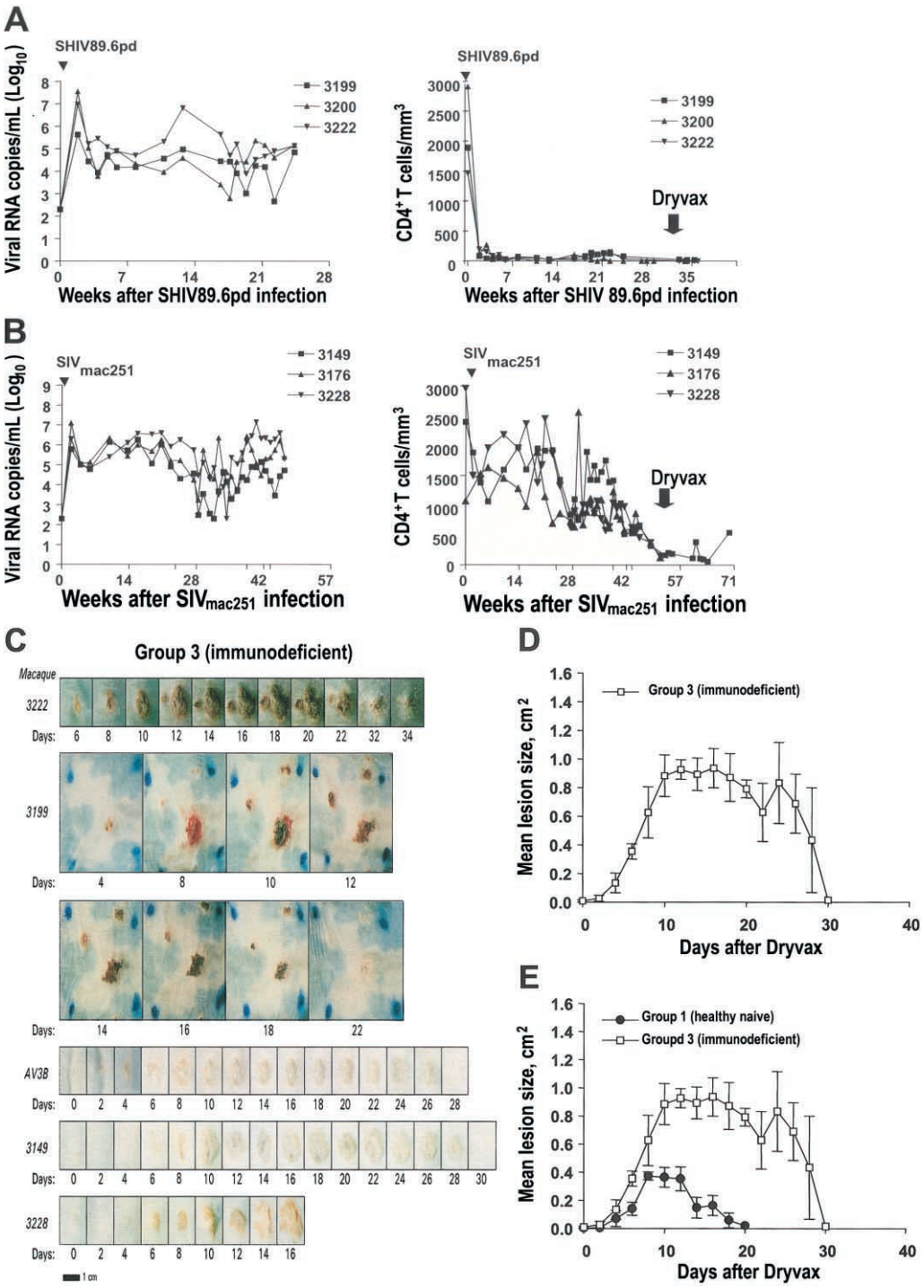

Figure 2. Dryvax-induced skin lesions, plasma virus levels, and CD4$^+$ T cell counts in immunocompromised macaques. *A,* Vaccinia-naive macaques infected with simian/human immunodeficiency virus (SHIV) 89.6 PD (group 3). Virus load is shown on the left, and CD4$^+$ T cell count is shown on the right. *B,* Vaccinia-naive macaques infected with simian immunodeficiency virus $(SIV)_{mac251}$ (561) [12] (group 3). Virus load is shown on the left, and CD4$^+$ T cell count is shown on the right. *C,* SHIV 89.6 PD–infected macaques (3222 and 3199) and SIV_{mac251}-infected macaques (AV3B, 3149, and 3228). *D,* Mean lesion size of macaques in group 3. *E,* Comparison of mean lesion size over time in macaques in groups 1 and 3.

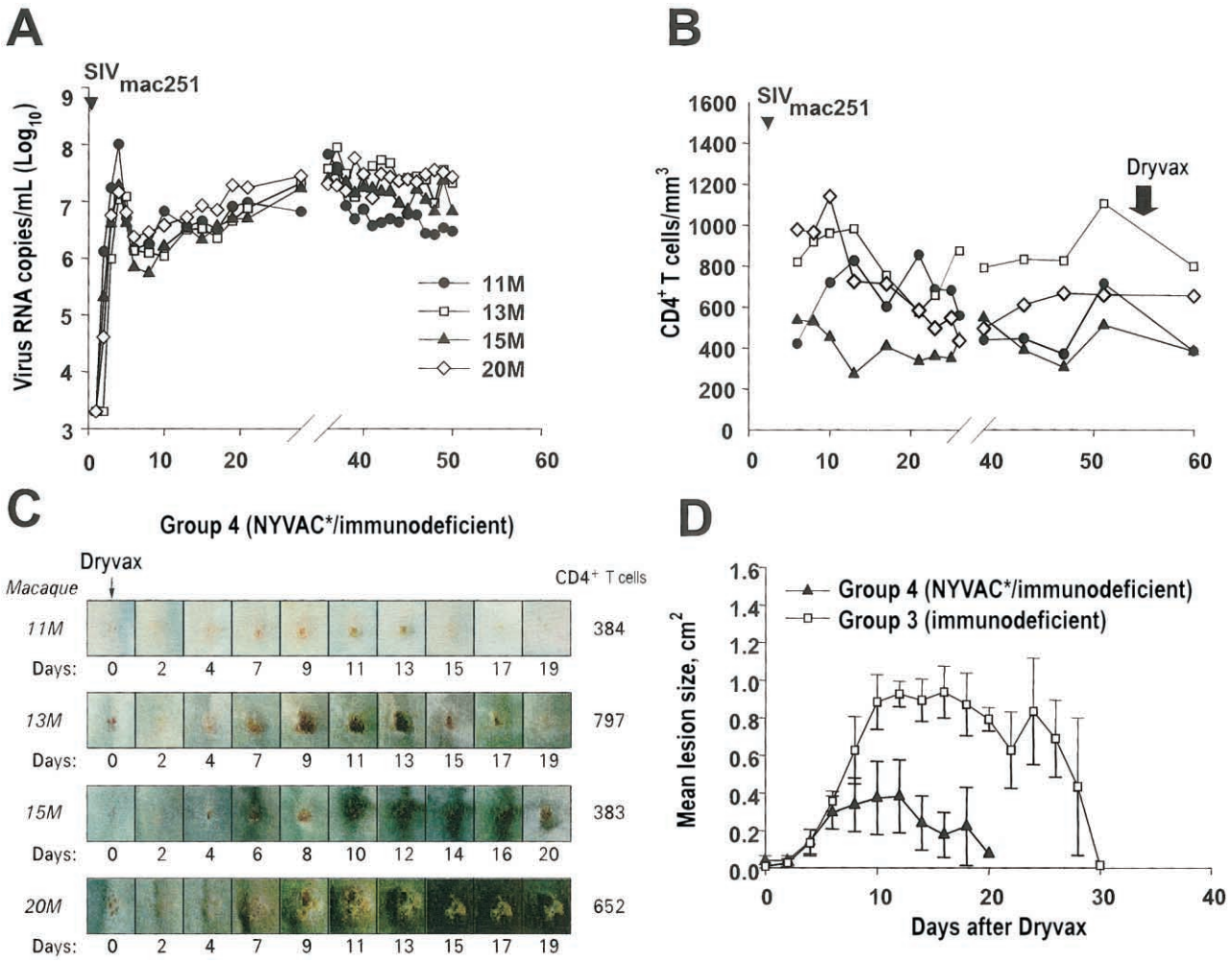

Figure 3. Virus loads, CD4+ T cell counts, and Dryvax-induced lesions in macaques vaccinated with NYVAC before simian immunodeficiency virus $(SIV)_{mac251}$ infection. Macaques vaccinated with NYVAC before SIV_{mac251} infection; *group 4 in table 2. *A* and *B,* Plasma virus level is shown on the left, and CD4+ T cell counts are shown on the right. *C* and *D,* Skin lesions in macaques previously vaccinated with NYVAC *(C)* and comparison of mean size of skin lesion between these macaques (group 4) and the vaccinia-naive immunocompromised macaques from group 3 *(D).*

pletion decreases time to resolution of Dryvax-induced lesions. In the possible scenario of a smallpox outbreak, Dryvax vaccination may pose safety concerns for a large number of vaccinia-naive, HIV-1–infected individuals. In the studies described above, macaques were vaccinated with NYVAC before immune-system damage that follows SIV_{mac251} infection [23]; therefore, we investigated whether vaccination with NYVAC after an established lentivirus infection would be equally effective in lessening the severity of Dryvax-induced lesions. Four macaques infected with SIV_{mac251} [17, 18] and 4 macaques infected with SHIV 89.6 PD (groups 5 and 6, respectively) were vaccinated with 3 inoculations of NYVAC and challenged with Dryvax. Macaques in groups 5 and 6 had CD4+ T cell counts of 360 ± 131 and 372 ± 160 cells/mm^3, respectively (table 2). After Dryvax challenge, the skin lesions in macaques in group 5 continued to enlarge (figure 4*B*) and reached a mean maximum lesion size of 0.8 ± 0.06 cm^2, which did not differ from that observed in macaques in group 3 (table 2). Nevertheless, the time to resolution was shorter in macaques in groups 5 and 6 than in macaques in group 3 ($P = .063$, Wilcoxon rank sum test) (figure 4*C*). Macaques in group 6 experienced a significantly smaller mean lesion size and shorter time to resolution than did the nonimmunized, CD4+ T cell–depleted macaques in group 3 ($P = .032$ and $P = .016$, respectively) (figure 4*D*). Macaques in group 6 fared better than did macaques in group 5 and experienced a significantly shorter time to resolution ($P = .029$) (figure 4*E*). Although macaques in groups 5 and 6 received an equal number of NYVAC immunizations, the lag between the last immunization with NYVAC and Dryvax exposure was much shorter in macaques in group 6 than in those in group 5 (6 vs. 12–36 months, respectively) (table 2), suggesting that a shorter lag between NYVAC and Dryvax vac-

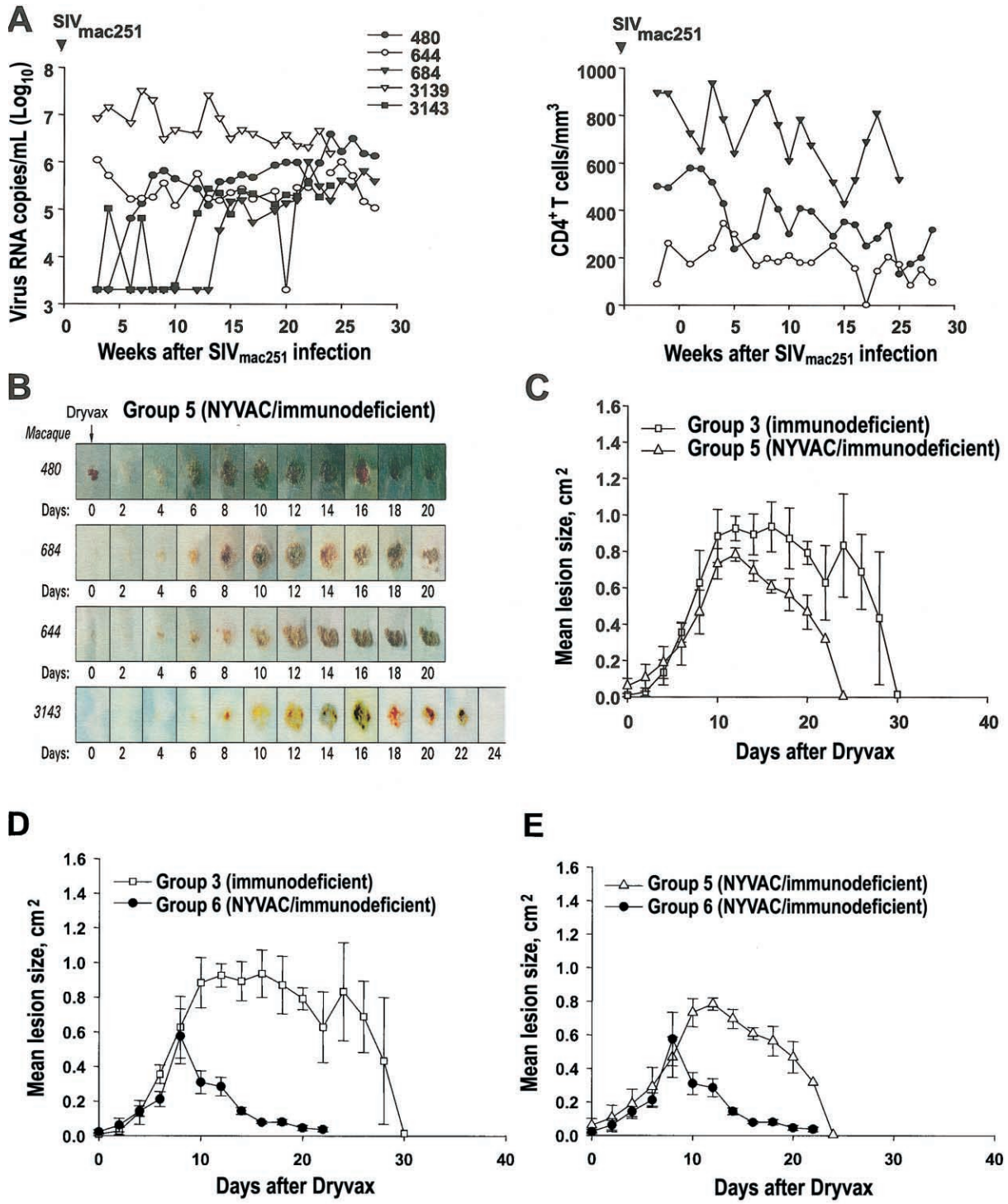

Figure 4. Virus loads, CD4+ T cell counts, and Dryvax-induced skin lesions in macaques vaccinated with NYVAC during an established simian immunodeficiency virus $(SIV)_{mac251}$ infection. *A,* For macaques in group 5, plasma virus level is shown on the left, and blood CD4+ T cell counts are shown on the right. *B,* Skin lesions and mean size of skin lesions over time. *C,* Comparison of macaques in group 3 (vaccinia naive) and group 5 (vaccinated with NYVAC after SIV infection). *D* and *E,* Mean lesion size in macaques in groups 3 and 6 *(D)* and in groups 5 and 6 *(E)* is depicted in the graphs.

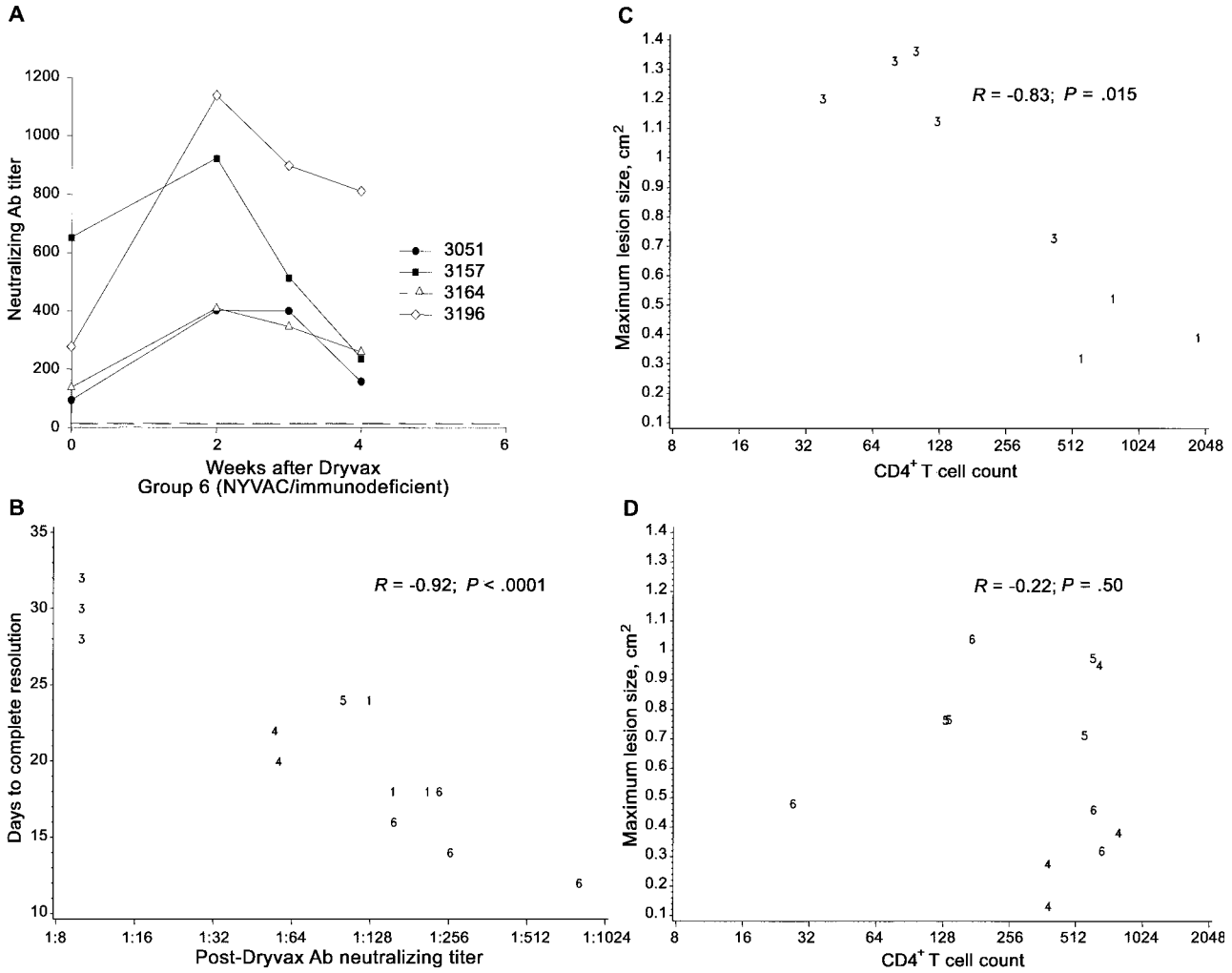

Figure 5. Immunological correlates of containment of Dryvax replication. *A,* Kinetics of neutralizing-antibody (Ab) titers (ID_{50} values) within the first 4 weeks after Dryvax challenge in macaques in group 6. *B,* Inverse correlation between neutralizing-antibody titers elicited within the first weeks of Dryvax challenge and time to resolution of skin lesions in macaques in groups 1, 3, 4, 5, and 6. *C* and *D,* A significant inverse correlation between $CD4^+$ T cell count and the maximum lesion size in vaccinia-naive macaques in groups 1 and 3 *(C)* and no correlation in vaccinia-experienced macaques *(D).*

cination in severely immunocompromised macaques lessens the severity of skin lesions.

Neutralizing-antibody titers to vaccinia inversely correlate with time to lesion resolution. The fact that previous vaccination of macaques, with NYVAC, was associated with faster resolution of Dryvax-induced lesions suggests that vaccinia-specific immune responses play a role in resolution of lesions. Neutralizing-antibody titers to vaccinia have been used as a measure of smallpox vaccination–induced immunity [24] and have been demonstrated to play a role in restriction of Dryvax replication, because passive administration of neutralizing antibodies has been reported to have a beneficial effect on decreasing the dissemination of vaccinia lesions [2, 3]. Plasma from 5 vaccinia-naive macaques had a background neutralizing activity of up to 1:15–1:27 in our assay (data not shown). After Dryvax challenge, antibody titers to vaccinia markedly increased in all macaques previously vaccinated with NYVAC (figure 5*B*), and an example of the kinetics of neutralizing-antibody appearance in immunocompromised macaques is presented in figure 5*A*, for macaques in group 6. Neutralizing antibodies to vaccinia developed within the first 5 weeks from Dryvax challenge also in all vaccinia-naive macaques in group 1 (by weeks 4 and 5, the titers were 1:213, 1:157, and 1:124, respectively). In contrast, macaques in group 3 did not develop vaccinia-neutralizing antibodies within the same period, indicating that the immunological damage induced by lentivirus infection delayed their ability to mount this humoral response (figure 5*B*).

To define the importance of vaccinia-neutralizing antibodies in restriction of Dryvax replication, a correlation analysis of the time to resolution of skin lesions and the neutralizing-antibody titer was performed. Indeed, a significant inverse cor-

relation between neutralizing-antibody titer and time to resolution of skin lesions was observed ($R = -0.92$; $P = .0001$) (figure 5*B*), indicating that neutralizing antibodies may be important in the restriction of Dryvax replication.

Correlation between absolute CD4⁺ T cell counts and maximum size of skin lesions in vaccinated and naive macaques. $CD4^+$ T cell counts and plasma-viremia levels in untreated HIV-1–infected individuals are predictors of development of opportunistic infections, including recurrence of latent viral infections [25]. Therefore, we investigated whether a correlation could be found between the degree of $CD4^+$ T cell depletion and the maximum size of Dryvax-induced skin lesions.

In the vaccinia-naive macaques in groups 1 and 3, which had a wide range of $CD4^+$ T cell counts (figure 2; table 1), the correlation of the $CD4^+$ T cell counts and lesion size was negative, and the Spearman's rank correlation coefficient ($R = -0.83$; $P = .015$) suggested an increased risk for excessive Dryvax replication with decreasing $CD4^+$ T cell counts (figure 5*C*). Interestingly, in the vaccinia-experienced, immunocompromised macaques in groups 4–6, this direct correlation was abrogated ($R = -0.22$; $P = .5$) (figure 5*D*), suggesting that vaccination with NYVAC before or after the onset of immunodeficiency may compensate for the loss of $CD4^+$ T cells. It is possible that the immunization-induced, vaccinia-neutralizing antibodies compensate for the difference in T cell response.

DISCUSSION

The replication competence of live vaccines, such as the only currently available smallpox vaccine, Dryvax, may pose safety concerns when injected in individuals with congenital, acquired, or iatrogenic immunodeficiency [6]. Because the number of patients with immunodeficiency has increased worldwide as a result of the HIV-1 epidemic, the increase in the number of organ transplants, and aggressive chemotherapy in patients with cancer, the risks associated with Dryvax vaccination may affect a larger portion of the population than before.

Here, we have modeled in immunocompromised macaques the effect of Dryvax vaccination and have found that life-threatening Dryvax skin lesions were less severe than expected. We also investigated whether previous vaccination with attenuated poxviruses, such as NYVAC, could lessen the adverse effects of Dryvax. The size and time to complete resolution of Dryvax-induced skin lesions and the development of vaccinia-neutralizing antibodies were parameters monitored in all 25 macaques studied.

Immunization with NYVAC before infection with lentivirus causes immunodeficiency-restricted Dryvax replication. Of importance, vaccination with NYVAC after the onset of $CD4^+$ T cell depletion also resulted in shorter time to resolution of skin lesions. A shorter interval between NYVAC and Dryvax vaccinations resulted in a further decreased mean size of and faster resolution of skin lesions. Thus, in the context of established immunodeficiency, a prime-boost approach with NYVAC and Dryvax within a short period of time may be safe. We have not assessed whether fewer, or even only 1, NYVAC immunizations preceding Dryvax exposure by 4–5 weeks would also be safe.

An inverse correlation was found between $CD4^+$ T cell counts and the size of Dryvax-induced lesions, suggesting that the degree of immunosuppression may correlate with the severity of Dryvax complications. Interestingly, however, in NYVAC-vaccinated macaques that developed vaccinia-neutralizing antibodies, the correlation between $CD4^+$ T cell counts and lesion size was negated, suggesting, on the one hand, that previous vaccination with NYVAC provides benefit in a manner at least partially independent of the $CD4^+$ T cell count and, on the other hand, that neutralizing antibodies induced by previous vaccination are key in the restriction of Dryvax replication, as also suggested by recent experiments in immunized rodents [26].

Local lesion formation after intradermal vaccinia inoculation is thought to be mediated by cell-to-cell transmission of the cell-associated enveloped virus and the extracellular enveloped virus. These forms of the virus were shown to be relatively resistant to antibody neutralization [27]. Therefore, it is possible that $CD8^+$ T cells play a role in limiting lesion size, whereas neutralizing antibodies prevent systemic spread of the virus (i.e., block viremia). $CD4^+$ T cells are required for both cytotoxic T cells and B cell responses. However, although they play a crucial role in the primary immune response to viral exposure, memory B cells are less dependent on $CD4^+$ T cell help, which may explain the significant antibody recall responses in immunocompromised macaques previously vaccinated with NYVAC (ID_{50} titer range, 1:95–1:899).

The immunocompromised macaques studied here were vaccinated with NYVAC at 6 months to a maximum of 36 months before Dryvax challenge, suggesting that this vaccine is able to induce lasting immune responses even as $CD4^+$ helper T cells are progressively depleted. However, the lag between NYVAC and Dryvax vaccinations appears to be important.

Although the data presented here demonstrate the feasibility of using NYVAC or other attenuated poxviruses, such as modified vaccinia virus Ankara [28–30], in already immunocompromised macaques, they also suggest that vaccination with these viruses after the onset of immunodeficiency may require careful modeling. Of importance, an essential goal is to assess whether lessening Dryvax replication by previous vaccination with attenuated poxviruses maintains the protective effect of Dryvax against smallpox. Exposure of macaques immunized with the NYVAC-Dryvax prime-boost approach to monkeypox [31] may yield useful information on the preservation of ad-

equate protective immunity to contain replication of this highly pathogenic poxvirus [31].

Acknowledgments

We thank Christopher Allen and Cindy Dougherty (Centers for Disease Control and Prevention), for providing Dryvax; Doug Yocom (Precision Medical), for providing bifurcated needles; Scott Cairns and Nancy Miller (National Institutes of Allergy and Infectious Diseases), for providing some of the infected macaques; Sharon Orndorff (Advance BioScience Laboratories), for coordination of the study; and Douglas R. Lowy (National Cancer Institute), for helpful discussion.

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